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A SYSTEM of genetic transduction has been discovered in the sexually fertile K-12 strain of *Escherichia coli*. This transduction is mediated by lambda, a temperate phage for which K-12 is normally lysogenic.

The distinctive features of the lambda-K-12 system include the following: (1) The transductions are limited to a cluster of genes for galactose fermentation. The *Gal* loci are closely linked to each other and to *Lp*, the locus for lambda-maintenance. (2) The transducing competence of lambda depends on how it is prepared. Competent lambda is produced by induction of lysogenic bacteria; lambda harvested from infected, sensitive hosts is incompetent. (3) The transduction clones are often heterogenotic, that is, heterozygous for the *Gal* genes which they continue to segregate. Technical advantages of the lambda system include recombinational analysis by the sexual cycle and the availability of lysates in which nearly every lambda particle is competent.

MATERIALS AND METHODS

Cultures

The origin and history of the *Escherichia coli* K-12 cultures studied have already been described (E. LEDERBERG 1950, 1952; LEDERBERG and LEDERBERG 1953). The emphasis will be placed here on the *Gal* loci (+ = fermenting galactose; − = nonfermenting) and on the locus which controls the maintenance of lambda (Lp_1).

The phenotypes of cultures with different alleles of Lp_1 are as follows:

	Lysed by lambda	Lyses Lp_1^s culture
Lp_1^s culture (sensitive)	yes	no
Lp_1^+ culture (lysogenic)	no	yes
Lp_1^r culture (immune)	no	no

Regardless of their Lp_1 genotype, cultures have been found to adsorb lambda. Thus Lp_1^+ and Lp_1^r are resistant to lysis by lambda in spite of their ability to adsorb the phage. In contrast with this, mutants resistant to lambda-2, a virulent mutant of lambda, are resistant because they do not adsorb either lambda or lambda-2 under the experimental conditions used here.

Media

The media used include: broth, Difco penassay; agar for phage assay, Difco nutrient agar with 0.5 percent NaCl; indicator medium, EMB agar plus one percent

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[2] Predoctoral research fellow of the National Science Foundation, 1953–54.

sugar; minimal agar, D(O); and minimal indicator agar, EMS (J. LEDERBERG 1950). Special supplements were added where indicated. All dilutions of phage lysates were made in either penassay or nutrient saline broth, and cell suspensions were diluted in either 0.5 percent saline or penassay broth.

General methods

Plates and tubes were incubated at 37°C. When high cell densities were desired, broth cultures were aerated by bubbling filtered air through them. Propylene glycol monolaurate (Glyco Products Co., Inc.) at a final concentration of 0.01 percent was added to bubbled cultures to lessen foaming. Phage assays were made either in agar layer or by spreading a portion of dilute lysate with *Gal*$^-$ cells on EMB galactose agar.

Lysates containing lambda in high titer were prepared by two methods: (1) "Induced lambda" was liberated from lysogenic bacteria after treatment with ultraviolet (UV) (WEIGLE and DELBRÜCK 1951); (2) "Lytic lambda" was harvested from sensitive bacteria infected with free lambda. The induced lambda was prepared as follows: aerated, penassay grown cultures of an *Lp*$^+$ strain (ca. 10^9 cells per ml) were sedimented in the centrifuge, the broth discarded, and the cells resuspended in 0.5 percent saline. The cell suspensions (10 ml) were exposed to the radiation from a GE Sterilamp (45 seconds at 50 cm) in open petri dishes on a platform shaker. After irradiation the suspensions were diluted with an equivalent volume of double strength penassay broth and aerated at 37°C until maximal clearing was obtained. This usually required from 2 to 3 hours. To produce lytic lambda, an inoculum of induced lambda was adsorbed on to penassay grown sensitive cells. After the adsorption period the cells were sedimented to separate them from the penassay broth and resuspended in nutrient saline broth. The suspension was then aerated until maximal clearing was obtained (4–5 hours). Induced lysates have phage plaque titers of about 3×10^{10} particles per ml, while lytic lysates have about 10^{10}.

Induced lambda was used in all experiments unless otherwise stated.

Methods for testing for transduction

In order to detect infrequent genetic changes, selective agar media were used: EMB agar for fermentation markers; EMB agar plus 100 micrograms per ml streptomycin for streptomycin resistance; minimal agar for nutritional markers. About 10^8 mutant cells in 0.1 ml broth or saline, and 0.1–0.2 ml of lysate were added to the surface of each agar plate and then spread with a bent glass rod. The plates were incubated 2–3 days before being scored.

Transduction clones selected by these methods develop in a heavy background of unchanged cells. On EMB medium, negative cells grow at the expense of the peptone; by using sugar as well, positive clones form papillate outgrowths from the negative background. EMB agar serves as an indicator as well as a selective medium; isolated positive colonies are deeply colored, while negative colonies remain translucent (illustrated in fig. 3).

The transduction clones were purified by the following procedure. Papillae were picked with a needle and suspended in 1 ml of sterile water. A loopful (ca. 0.001

ml) of this suspension was then streaked upon a portion of another plate of the EMB agar. These primary dispersals of the transduction clones were nearly always mixed. Direct picking and streaking, or spotting without any purification cannot be trusted. From the primary streaks a single colony that looked pure was picked to water and streaked as before. This operation was repeated once again, and a single colony from the last streaking was taken to represent the transduction clone. In addition to freeing the transduction clone from unchanged background cells, this method of purification may also act selectively within an unstable clone. Picking apparently pure colonies leads to an overestimate of the fraction of non-segregating clones.

RESULTS

The transductions

Although a number of different loci affecting diverse portions of the genotype were tested, only genes of a cluster of loci for galactose fermentation were transduced by lambda lysates (MORSE 1954). The *Gal* loci, of which about seven have been investigated thus far, are closely linked to one another (less than one percent recombination) and to Lp_1, the locus for lambda maintenance (LEDERBERG and LEDERBERG 1953, and unpublished).

The transformation of Gal^- cells to Gal^+ by induced lambda is illustrated in figure 1. Each papilla is a clone of galactose fermenting cells; on the area of the plate to which lysate was added, most of the Gal^+ papillae are transduction clones. The

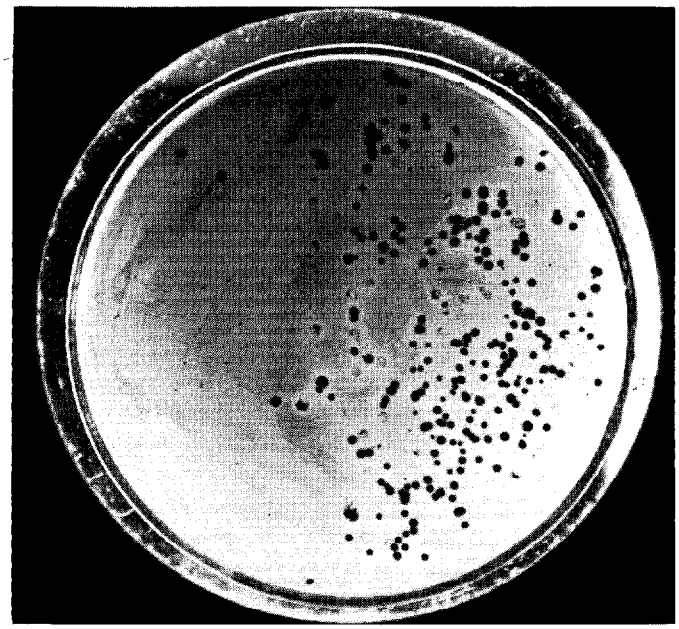

FIGURE 1.—The production of Gal^+ papillae from a Gal^- background of cells by a lambda lysate. Left, the control, no lysate added. Right, 0.1 ml of lysate from a Gal^+ culture. Some of the papillae have been picked with a needle.

TABLE 1

Transformation of Gal$^-$ *cultures by lysates of* Gal$^+$

Recipient culture	Lysate of:	Lambda titer in 10^{10} per ml	Number of *Gal*$^+$ papillae		*Gal*$^+$ papillae per 10^7 lambda
			Control (no lysate)*	0.1 ml lysate	
Gal$_1^-$ *Lp*$^+$	*Gal*$^+$	1.4	2	405	2.9
	Gal$_1^-$	2.4	2	2	—
Gal$_2^-$ *Lp*$^+$	*Gal*$^+$	1.4	20	356	2.4
	Gal$_2^-$	4.9	20	10	—
Gal$_4^-$ *Lp*$^+$	*Gal*$^+$	1.4	47	394	2.5
	Gal$_4^-$	1.7	47	50	—
Gal$_4^-$ *Lp*^{s}	*Gal*$^+$	1.4	4	2112	15.1
	Gal$_4^-$	1.7	163	86	—
Gal$_4^-$ *Lp*^{s}†	*Gal*$^+$	2.3	10	3020	13.1
	Gal$_4^-$	1.7	10	18	—
Gal$_4^-$ *Lp*^{s}†	*Gal*$^+$	2.3	5	1296	5.6
Gal$_4^-$ *Lp*^{r}	*Gal*$^+$	2.3	40	161	0.5
Gal$_4^-$ *Lp*^{r}‡	*Gal*$^+$	1.4	29	129	0.7
Gal$_4^-$ *Lp*^{r}‡	*Gal*$^+$	1.6	28	92	0.4

* The *Gal*$^+$ papillae on the control are spontaneous reversals of phenotype.

† Different stocks.

‡ Different experiments.

quantitative relationships are illustrated in figure 2. The data can be summarized: (1) Regardless of the *Lp*$_1$ genotype of the recipient, transductions were obtained; (2) with each genotype the number of transductions was proportional to the amount of lysate plated; (3) *Lp*^{s} recipient cultures gave 5 to 10-fold more papillae per unit of lysate than either *Lp*$_1^+$ or *Lp*$_1^r$. Further, the transducing activity of lysates (which contain 10^{10} lambda per ml) varies according to the number of cells plated: (1) with *Lp*$_1^+$ *Gal*$^-$ cultures there is a two-fold increase between 10^6–10^9 cells per plate, with a plateau of maximal yield around 10^8 cells per plate; (2) *Lp*^{s} *Gal*$^-$ recipient cultures show about a six-fold increase over a similar range of cell platings, with the highest yield at the highest cell density.

The transducing activity of lysates is specific; that is, a lysate of a *Gal*$_x^-$ culture will not transform *Gal*$_x^-$ cultures (table 1) but *Gal*$^+$ papillae were found with a *Gal*$_y^-$ culture. The specificity is extended further in that some galactose positive phenotypic reversals of a *Gal*$^-$ culture give lysates with transducing activity on the original *Gal*$_x^-$ indicator (table 2). The different types of phenotypic reversals may be understood under the following hypothesis: (1) reverse mutations (*Gal*$_x^-$ to *Gal*$_x^+$) yield cultures that give active lysates, and (2) suppressor mutations (*Gal*$_x^-$ Suppressor$^-$ to *Gal*$_x^-$ Suppressor$^+$) will yield incompetent cultures when the suppressor lies outside the region transduced.

From the data in table 1 and figure 2 the ratio of the transducing particles to the lambda particles in a lysate may be obtained. *Lp*^{s} recipient cultures give about one transduction per 10^6 lambda; *Lp*$^+$ recipients, one per 10^7. One per 10^6–10^7 lambda will be referred to as LFT (low frequency of transduction).

TABLE 2

The action of lysates of reverse mutants

Lp^+ recipient culture	Lysate of reversion	Numbers of Gal^+ papillae observed	
		Control (no lysate)	0.1 ml lysate
Gal_1^-	Gal_1^+ #1	0	648
Gal_2^-	Gal_2^+ #1	10	96
	Gal_2^+ #2	6	552
Gal_4^-	Gal_4^+ #5	39	204
	Gal_4^+ #8	25	291

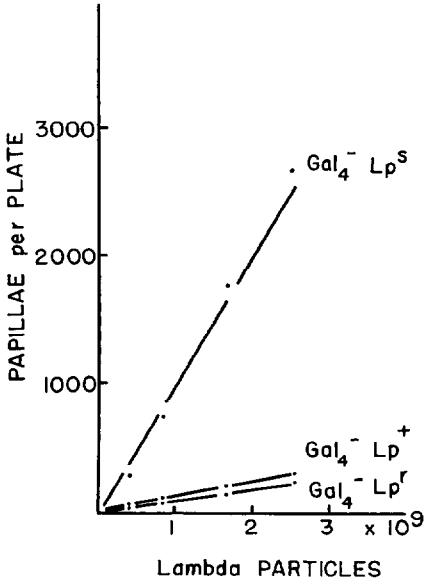

FIGURE 2.—Proportionality between amount of lambda lysate (LFT) plated and number of papillae formed from Lp^s, Lp^+ and Lp^r Gal^- cultures. The ratio of papillae to lambda particles is 10^{-6} for an Lp^s culture, 10^{-7} for Lp^+ and Lp^r cultures.

Examination of the Gal+ clones formed by transduction

After purification the transduction clones were examined for changes at loci other than the *Gal* series. A number of markers were examined, including fermentative, nutritional, and phage and drug resistance mutations. The only changes at other loci were Lp^s to Lp^+ in lambda sensitive recipients, and occasionally Lp^r to Lp^+ in lambda immune cultures. Transduction clones from Lp^+ recipients were invariably Lp^+.

To determine whether lysogeny was causally related to transduction, a reconstruction experiment was done. To a mixture of lysate and Gal^- Lp^s cells, Gal^+ Lp^s cells labelled with a mutant character were added to estimate the frequency of chance lysogenization in the untransformed cells in a transduction mixture. After papillae had formed, they were picked, purified, and on the basis of the differential label, divided into: (1) the inserted Gal^+, and (2) the transductions. The frequency

TABLE 3

Comparison of the lysogenization of transformed and non-transformed sensitive strains: reconstruction experiment

Types recovered from mixture* of Lp^s bacteria and LFT lysate	Number of clones examined	Percent of clones lysogenized
Inserted $Gal^+Lac^+S^s$	46	68.5
Recipient $Gal^-Lac^-S^r$ (non-transformed)	40	72.5
Transduction $Gal^+Lac^-S^r$	103	100.

* 10^8 $Gal^-Lac^-S^r$, 100 $Gal^+Lac^+S^s$, 10^9 lambda particles.

of lysogeny was determined in the two classes, and in the Gal^- background. Whereas unchanged Gal^- cells and the inserted Gal^+ were each only 70 percent lysogenized, the transduction clones were 100 percent lysogenized (table 3).

When Lp^r cultures were used as recipients, 14/112 (12 percent) of transduction clones formed were Lp^+. Although the fraction is small, all previous attempts to lysogenize these cultures have been unsuccessful. The isolation of transduction clones evidently selects for these cells that have been infected with lambda particles from the input lysate.

The original Gal^+ strain and spontaneous reversions of the Gal^- mutants have all been stable in ordinary culture. However, the Gal^+ clones formed by transduction are unstable for galactose fermentation as shown by the recurrence of negative and mosaic colonies (fig. 3). Despite many serial single colony isolations the galactose-positives continue to segregate galactose negative progeny. They behave as if

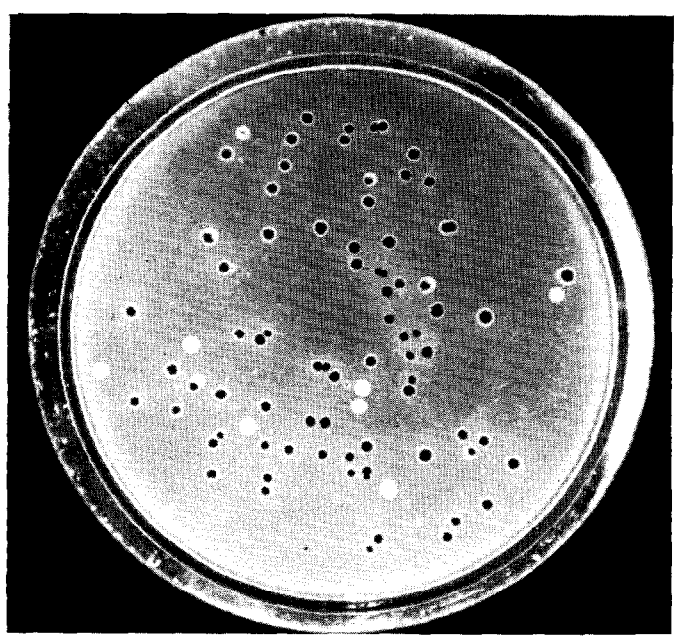

FIGURE 3.—EMB galactose agar plate spread with cells form a culture of a heterogenote, showing Gal^+, Gal^- and sectoring colonies.

TABLE 4

Frequency of instability for galactose fermentation among the transduction clones

Recipient cells	Unstable clones/total examined	Percent unstable
Gal_1^- Lp^s	9/22	41
Gal_1^- Lp^+	40/48	83
Gal_2^- Lp^+	22/24	92
Gal_4^- Lp^s	13/24	54
Lp^+	20/24	83
Lp^r	29/48	60
Gal_6^- Lp^s	6/8	75
Gal_8^- Lp^s	28/48	58
Lp^+	16/24	67

heterozygous for a single gene (or short chromosome segment) and may be designated as heterogenotes. Instability among the transduction clones is quite frequent; 484 of 609 clones (70 percent) were found unstable (representative data are given in table 4). This estimate is probably low because the purification procedure acts selectively against unstable clones.

The frequency of segregation has been estimated from the incidence of Gal^- in small clones of heterogenotes. The probability of segregation per bacterial division is about 2×10^{-3} (table 5). By repeated reisolation, however, heterogenotic lines can be maintained indefinitely.

The segregants from the heterogenotic clones were examined with regard to their *Gal* and *Lp* character. Lysates of the segregants have no transducing activity on the Gal^- culture that was used as the recipient in forming the transduction clone and are therefore allelic to it. The same lysates continue to give one transduction per 10^6–10^7 lambda (LFT ratio) on non-allelic Gal^- cultures. With different recipient cultures the *Lp* alleles of the segregants were (1) Lp^+ recipient, all segregants Lp^+; (2) Lp^s recipients, all segregants Lp^+; (3) Lp^r cells, the segregants were usually Lp^r. In one instance, a heterogenote segregated both Lp^+/Lp^r and Gal^+/Gal^-.

Lysates prepared from the heterogenotes have two outstanding features: (1) instead of containing 10^{10} lambda particles per ml, they seldom have titers higher than 5×10^8, particularly if they originate from cultures containing few Gal^- segregants; (2) the number of transducing particles in these lysates is often nearly equal to the number of lambda particles in the lysate (table 6). These lysates will be referred to as HFT (giving a high frequency of transduction).

Transductions with lysates of heterogenotes

Platings of highly diluted HFT lysate with Lp^s and Lp^+ bacteria give a number of papillae. The number of papillae obtained with Lp^s cells is, however, less than that obtained with Lp^+. The lower yield with Lp^s recipients may result at least in part from the loss of potential transductions through lysis of the recipient cell or of some of its early progeny.

With HFT lysates it is possible to transform a large fraction of a cell population, and to observe transduction without strong selection. By adsorbing HFT lambda onto cells, diluting and plating on EMB galactose to obtain well isolated colonies

TABLE 5

Frequency of segregation from the heterogenotes

Heterogenote	Test clones*			Probability of segregation per 10^3 bacterial divisions†
	Number of cells in inoculum	Number of *Gal*$^-$ cells	Total cells	
Gal$_1^-$_//*Gal*$^+$	2.1‡	6	1169	1
		3	595	1
		4	251	4
		23	1252	3.6
		9	1113	2
		19	897	4.3
		103	2750	6.6
		319	1622	36.8
		22	1966	2.0
		0	237	0
Gal$_2^-$_//*Gal*$^+$	1.5§	11	323	8.1
		2	176	3
		8	1669	0.9
		3	317	2
		52	1236	8.2
		0	10	0
		36	1055	6.7
		3	299	2
		6	386	4
		55	1965	5.1

* A fully grown culture in penassay broth was diluted to give about 10 cells per ml. Twenty samples of 0.1 ml were taken up in 0.2 ml serological pipettes which were supported in a horizontal position on a tray. The pipettes were incubated at 37°C for 4.5 hours. Each pipette was then blown out on to an EMB galactose agar plate, and the inside of the pipette washed with 0.1 ml of broth. The washing was added to the plate, and the inoculum spread with a glass rod. After 18 hours incubation at 37°C the number of *Gal*$^+$ and *Gal*$^-$ colonies on the plates was determined.

† Using the equation $a = 0.602r/N \log N$, (modified for the indicated units from LURIA and DELBRÜCK 1943) where r = the number of *Gal*$^-$ segregants and N = the clone size. The probability of segregation is also estimated by the fraction of cultures containing no segregants.

$$a = \frac{2.3}{N} \log \frac{1}{P_0} (P_0 = \text{fraction of cultures with no segregants.})$$

In the first experiment, using $N = 2^{10}$

$$a = \frac{2.3}{1024} \log 1/1/19 = 2.8 \times 10^{-3}$$

In the second experiment, using $N = 2^{10}$

$$a = \frac{2.3}{1024} \log 1/1/11 = 2.6 \times 10^{-3}$$

‡ The assay plates showed this culture to have *Gal*$^+$: *Gal*$^-$ in the ratio 106:4. Of the twenty samples in this experiment, one contained only *Gal*$^+$, one contained only *Gal*$^-$, and 18, both *Gal*$^+$ and *Gal*$^-$. Only the plates that were counted are given. Nine plates were too crowded to be counted accurately.

§ The ratio of *Gal*$^+$:*Gal*$^-$ in the parent culture was 128:19. The twenty cultures were distributed as follows: failed to grow, 9; contained only *Gal*$^+$, 1; contained both *Gal*$^+$ and *Gal*$^-$, 10. One plate had approximately equal numbers of *Gal*$^+$ and *Gal*$^-$ and was assumed to have come from a mixed inoculum.

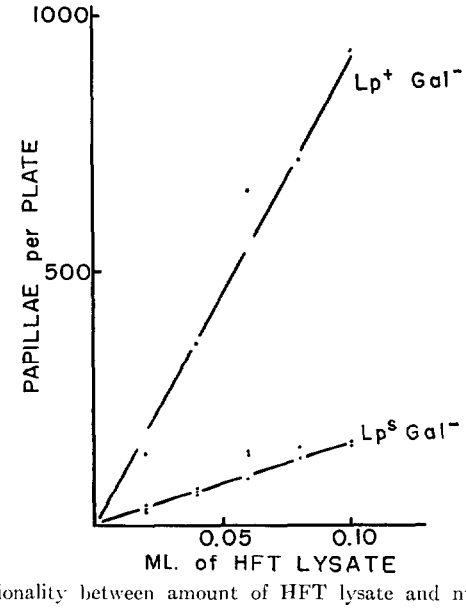

FIGURE 4.—Proportionality between amount of HFT lysate and number of papillae formed from Lp^s and Lp^+ Gal^- cultures. For the assays, a lysate containing 1.6×10^8 phage/ml was diluted a thousandfold.

it is possible to study individual transduction clones derived from single particle infections of isolated bacteria. At the optimal ratio of about 10 lambda particles per cell, the fraction of cells transformed ranged from 5 to 15 percent.

Evidence that lambda is the vector of transduction

That lambda is the vector of Gal transduction is suggested by previous experiments: (1) the 100 percent lysogenization of Lp^s recipients by LFT lysate transductions; (2) the incidence of lysogenicity in transduction to Lp^r recipients. In

TABLE 6

The high frequency of transduction (HFT) given by lysates of heterogenotes

Heterogenote	Lysate of the heterogenote		
	Lambda particles per ml	Transductions per ml	Transductions per lambda particle
Gal_1^-_//Gal^-	1.2×10^8	2.1×10^7	1/5.7
Gal_1^-_//Gal^+	5.8×10^8	1.8×10^7	1/32*
Gal_2^-_//Gal^-	5.4×10^7	3.6×10^7	1/1.5
Gal_2^-_//Gal^+	7.6×10^7	4.2×10^7	1/1.8
Gal_4^-_//Gal^+	1.5×10^8	7.4×10^7	1/2.0
Gal_4^-_//Gal^+	7.3×10^8	2.5×10^7	1/29*

* With the exception of these cases, the cultures used for making the lysates were started from a single apparently pure Gal^+ colony on EMB galactose. The lower ratio in the exceptional cases, and the higher lambda titer is probably the result of the presence in the source cultures of a larger number of Gal^- segregants. Assay of the transductions was made with Lp^- cells.

TABLE 7

Failure of transduction to lambda-2 resistant mutants

Recipient cells (Lp^+)	Lambda-2 reaction*	Number of fermenting clones	
		No lysate added	0.1 ml of Gal^+-lysate
Gal_1^-	sensitive	1	426 (LFT)
	resistant	1	2
Gal_2^-	sensitive	20	356
	resistant	14	14
Gal_4	sensitive	89	296
	resistant	50	57
Gal_1^-	sensitive	2	10^7 (HFT)
	resistant	3	4

* Lambda-2 resistant mutants do not adsorb lambda or lambda-2.

addition, lambda and the transducing agent are adsorbed to about the same degree by Lp^s cells, and both are inactivated by crude anti-lambda serum. More definite evidence was the failure of lambda-2 resistant cells to adsorb either lambda or transducing activity or to be transformed even by HFT lysates (table 7).

Conclusive evidence that lambda is the vector of transduction is found from the behavior of single transduction clones: (1) Heterogenotes formed from HFT lysate and Lp^s cells at low lambda multiplicity are always either overtly lysogenic (Lp^+) or carry a defective prophage (Lp^r) (table 8). (2) Proportionality between number of transductions and amount of lysate at high dilution (fig. 4). For a two-factor-system to be invoked at these dilutions, the accessory factor would have to exceed the lambda by at least 10^{10}, which would imply a concentration of this fancied element in undiluted HFT lysate of 10^{18} per ml, which should be compared with Avogadro's number.

Early segregation of Lp and Gal in transduction clones

HFT Gal^+ lambda was mixed with a culture of Gal_4^- bacteria to give 2.6×10^7 lambda and 7×10^8 cells per ml, a multiplicity ratio of 0.04. The suspension was then diluted and plated on EMB Gal to give about 100 cells per plate. After 24 hours incubation, on 7 plates, a total of 8 colonies with Gal^+ sectors was noted. Each of these colonies was sectored, with a large Gal^- component. Each colony was restreaked, and 20 to 30 Gal^- reisolated from each line. Of the 8 lines, the Gal^- from 3 gave only Lp^+, from 5 gave mainly Lp^s with a few Lp^+. Ten Gal^+ (heterogenote) colonies were also picked from each line. All of them were Lp^+ and of a total of 297 Gal^- segregants subsequently reisolated from these 60 heterogenotic colonies, all were Lp^+ also. The frequent segregation of Lp^+/Lp^s subclones from lambda-infected Lp^s cells has been noted previously (LEDERBERG and LEDERBERG 1953; LIEB 1953). The correlation of Lp^+ and Gal^+ evidently extended, in the 5/8 clones that segregated both markers, to the early intraclonal progeny. Since the heterogenotes do not continue to segregate Lp^s, these results are economically interpreted on the basis of the multinucleate character of the bacterial cells. The early segregation would represent the separation of unaltered Lp^s Gal^- nuclei from the

TABLE 8

Incidence of lysogenicity in isolated heterogenotes

1. *The transductions*

Gal⁻ cells exposed to:	Number of colonies observed		
	Unaltered *Gal*⁻	With *Gal*⁺	*Gal*⁻ partially lysed
Broth	3280	0	0
HFT lysate*	2801	31	54

2. *Examination of the colonies after exposure to HFT lysate*

Colony type	Number of colonies examined	Number of colonies		
		*Lp*ˢ	*Lp*⁺	*Lp*ʳ
Unaltered *Gal*⁻	31	31	0	0
With *Gal*⁺	26	0	23	3

* One ml of cell suspension (4.1×10^9 cells) was added to one ml of HFT lysate (1.2×10^9 plaques per ml, 3.0×10^8 transducing particles per ml) and the mixture incubated at 37°C for 10 minutes. The cells were then centrifuged down, the supernatant discarded, and the cells resuspended in one ml of broth. The suspension was then diluted and plated on EMB galactose agar. The tube contained 3.5×10^9 cells after HFT lysate exposure. 1.1 percent of exposed cells were transformed, and 1.8×10^7 transductions per ml were accomplished.

nucleus with which the prophage-*Gal*⁺ complex has associated. The segregation of *Gal* and stability of *Lp* in the heterogenotic subclones will be taken in later communications.

The failure to observe transduction with lytic lambda

The experiments described above employed UV induced lysates. That lytic lambda, prepared by the growth of lambda on sensitive cells is incompetent in transduction is evident from the following: (1) lytic lambda failed to augment the number of papillae when added to *Gal*⁻ cells on EMB galactose agar; (2) the occasional *Gal*⁺ clones that were found on plates to which lytic lambda was added were all stable and were presumably spontaneous reversions. The lysates used in these experiments were made by growing induced lambda from a Gal_4^- culture on a *Gal*⁺ culture, and the initial tests of competence of the lysates were made on Gal_4^- cultures. In this way, confusion by "carry-over" of the inoculum phage was avoided. The experiments were executed on a scale that should have detected as little as 3% of the activity per phage of LFT induced lysates.

Failure to observe transduction at loci other than Gal

Attempts with LFT, HFT, or lytic lysates to transduce genes at other loci were unsuccessful.

The unsuccessful tests for transductions of prototrophy to auxotrophic cultures involved: histidine; leucine (two loci); methionine; proline; glycine or serine; tryptophane.

The fermentation markers that were not transduced included: lactose (Lac_1); maltose (two loci); arabinose (two loci); xylose; glucose.

The attempt to transduce streptomycin resistance to sensitive cells was unsuccessful.

In the *E. coli* compatibility system, failure to transduce the following was noted: (1) by lysates of Hfr cultures, F^+ and F^- recipients to Hfr; and F^- recipients to F^+; (2) by lysates of F^+, F^- recipients to F^+.

The most extensive tests were made on genes at loci known to be linked to the *Gal* series (*Hfr*, histidine; CAVALLI-SFORZA personal communication, and proline), or mutations other than *Gal* (W435, Lac_3^-, LEDERBERG and LEDERBERG 1953 and some auxotrophs) which had occurred coincidently with changes of Lp^+ cultures to Lp^s.

In considering the transduction of specific loci, interactive effects should be kept in mind. For example, papillae were observed on EMB lactose, arabinose, and xylose, respectively, in tests with multiple marker stocks. When purified, however, these papillae were negative for the indicated sugar, but gave galactose-positive colonies. Historically, transduction papillae were first observed in platings of a treated Gal^- Lac^- culture on EMB lactose. The papillae proved to be Gal^+ Lac^- rather than Gal^- Lac^+. Evidently, all these sugars have slight selective potentials for Gal^+ clones.

Other observations

Most lambda lysates are viscous when first obtained. The viscosity is destroyed: (1) by DNAase, an indication that DNA is the cause of viscosity; (2) spontaneously at a slow rate. Exposure of lambda lysates to DNAase has not affected either transduction or plaque titers.

Transduction of the *Gal* gene is not restricted when either the donor or the recipient culture is (1) a prototroph or any of a variety of auxotrophs; (2) *Hfr*, F^+ or F^-, in any combination. Transduction is controlled (1) by the method of lysate production, and (2) the ability of the recipient cells to adsorb lambda. The only genes transduced are the *Gal* loci.

Gal^- mutants in *E. coli* strains other than K-12 that adsorb lambda can be transformed. As in strain K-12 the transformation does not require that the recipient be sensitive; among the susceptible strains are lambda sensitives, lambda immunes, and host modifiers of K-12 lambda (E. LEDERBERG 1954). However, lambda was incompetent when tested on galactose negative mutants of Salmonella, and transducing Salmonella phage (ZINDER and LEDERBERG 1952) failed to transform *E. coli*.

DISCUSSION

Galactose-negative cultures of *E. coli* are transformed to galactose-positive by certain lysates containing the phage lambda. That this process is genetic transduction by lambda particles is established by the following: (1) Gal_y^- cells are transformed to Gal^+ by lysates of Gal_y^+ cultures but not by Gal_y^-. (2) However, Gal_y^+ obtained by reversion regains its ability to transform the Gal_y^-, which emphasizes

the role of the donor genotype in effective transformation. (3) The transformed positives are unstable, and segregate Gal_y^- and not other galactose types. The various "Gal_y" used for these experiments include Gal_1, Gal_2, Gal_3, Gal_4, Gal_6, Gal_7 and Gal_8. (4) All transduction clones obtained from Lp^s recipients become lysogenic for lambda (either Lp^+ or Lp^r). (5) Transduction is not obtained with cells unable to adsorb lambda.

The contrasting features of the *E. coli*-lambda and the Salmonella systems of transduction are summarized as follows:

Range of genes transduced	*E. coli* K-12 phage lambda only *Gal*	Salmonella phage PLT22 any selectable marker
Localization of prophage	*Lp* locus linked to *Gal*	Unknown
Competence of lytic phage	No	yes
Transduction clones	unstable heterogenotes	stable
Efficiency of transduction, per phage	LFT 10^{-6} HFT 10^{-1}	10^{-5}–10^{-6}
Sexual fertility of the host	Fertile, subject to *F* compatibility system	Unknown

The two systems are alike in the following respects: (1) Genetic factors are carried by phage particles; (2) The specificity of the transducing particles is determined by the genetic content of the donor bacteria, in contrast to lysogenic conversions (UETAKE, ET AL. 1955); (3) The genetic material is inaccessible to DNAase and other enzymes; (4) Transduction occurs without regard (except for quantitative changes in yield) to the lysogenic or sensitive status of the recipient cells. In both systems UV induced phage is competent, but lytic phage is competent only in Salmonella.

However, the two systems evidently do not cross-react; lambda does not transform Salmonella and conversely, probably because of the specificity of phage adsorption.

Several of these features may be related in origin. For example, the limitation both on the mode of inclusion in the phage (i.e., only after induction of a lysogenic bacterium), and on the genetic material that can be transduced suggest that the physical proximity of the *Gal* loci to the prophage site determines transduction competence of lambda. This is supported by the linkage observed in crosses of *Lp* to *Gal*. Presumably the linked *Gal* genes may sometimes accompany the prophage into the maturing lambda particle when lysogenic bacteria are irradiated. The failure to obtain lambda particles with transducing activity when the phage is grown lytically on sensitive cells would be explained on this hypothesis, since the lambda may have no specific association with the *Lp-Gal* chromosomal segment during lytic growth.

The heterogenotic clones which result from transduction are isolated through the effectiveness of the *Gal* genes that accompany the prophage. In LFT transductions, this is a rare event; the HFT quality of lysates from heterogenotes may result in part from the prior selection of an effective fragment and its reproduction as such in the growth of the clone.

The persistence of the fragment in transduction clones requires an *ad hoc* explanation, possibly related to the presence of an *Lp* region in the fragment. For example, *Lp* might be closely linked to a centromere; it may function as a centromere itself; it may be adapted to synapse with the homologous site of an intact chromosome.

At any rate, the *Lp* region is singular in at least two respects: it is close to a regular point of breakage in crosses determined by *F* polarity (LEDERBERG and LEDERBERG 1953; NELSON and LEDERBERG 1954; CAVALLI-SFORZA and JINKS 1956) and the *Lp* segment (considered as prophage) is capable of independent replication as a phage. If comparable singular regions exist in Salmonella, they have not yet been revealed in the occurrence of heterogenotes.

The occurrence of sexual recombination and transduction in the same organism raises the technical question of their experimental confusion. Since sexual recombination requires intact cells, and transduction is accomplished with a cell-free lysate, sexual recombination can have no direct bearing on transduction experiments. Furthermore, although crossing is completely blocked between F^- cultures, the compatibility status has no effect on transduction. On the other hand, the rarity of LFT transduction makes it a priori unlikely that transduction will significantly interfere with segregation ratios in crosses.

Crosses of the various combinations of cultures carrying different *Lp* alleles will be presented in detail in further reports. However, they have indicated that combinations involving Lp^+ (where transduction could occur) do not give appreciably different frequencies of Gal^+ than $Lp^s \times Lp^s$ crosses (where lambda transduction is not possible). In addition, the Gal^+ prototrophic recombinants obtained are stable for galactose fermentation. Even crosses of known heterogenotes (capable of HFT lambda) have not given increased frequencies of Gal^+. These observations suggest that lambda transduction has not significantly affected results obtained by crossing.

The mosaic colonies of heterogenotic cultures (fig. 3) are reminiscent of those formed by segregating diploids of *E. coli* K-12. The latter, of course, are segregating blocks of many linked markers, not merely the *Gal* genes. Diploids are, however, more difficult to maintain without the benefit of balanced selective markers. They segregate twenty times as frequently as heterogenotes, as can be judged from the appearance of the colonies and from rates calculated from cell pedigrees (ZELLE and LEDERBERG 1952 and unpublished).

Further studies involving the use of two or more *Gal* markers, and relating transduction to sexual recombination analysis will be presented shortly, together with further consideration of the genetics of the prophage.

SUMMARY

Transduction of several Gal^+ genes from galactose positive (Gal^+) to galactose negative cells (Gal^-) by the bacteriophage lambda has been demonstrated. The resultant galactose positive clones have been found to be heterozygous for the *Gal* region and have been designated as heterogenotes ($Gal^-//Gal^+$). Segregation and the reappearance of Gal^- from the heterogenotes occurs about once per 10^3 bacterial divisions. The low frequency of lambda particles with *Gal* genes ($1/10^6$) from haploid cultures resembles other transduction systems. However, heterogenotic cultures

produce lysates in which nearly every lambda particle carries *Gal* genes. No other markers have been transduced by lambda, and the competence of lambda in transduction depends upon its production from lysogenic cells, rather than by lytic growth on sensitive bacteria.

LITERATURE CITED

CAVALLI-SFORZA, L. L., and J. L. JINKS, 1956 Studies on the genetic system of *E. coli* K-12. J. Genet. In press.

LEDERBERG, E., 1950 Genetic control of mutability in the bacterium *Escherichia coli*. Doctoral Dissertation, University of Wisconsin.

1952 Allelic relationships and reverse mutation in *Escherichia coli*. Genetics **37:** 469–483.

1954 The inheritance of lysogenicity in interstrain crosses of *Escherichia coli*. Genetics **39:** 978.

LEDERBERG, E., and J. LEDERBERG, 1953 Genetic studies of lysogenicity in *Escherichia coli*. Genetics **38:** 51–64.

LEDERBERG, J., 1950 Isolation and characterization of biochemical mutants of bacteria. Methods in Medical Research **3:** 5–22.

LIEB, M., 1953 The establishment of lysogenicity in *Escherichia coli*. J. Bacteriol. **65:** 642–651.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MORSE, M. L., 1954 Transduction of certain loci in *Escherichia coli* K-12. Genetics **39:** 984.

NELSON, T. C., and J. LEDERBERG, 1954 Postzygotic elimination of genetic factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U. S. **40:** 415–419.

UETAKE, H., T. NAKAGAWA, and T. AKIBA, 1955 The relationship of bacteriophage to antigenic changes in salmonellas of group E. J. Bacteriol. **69:** 571–579.

WEIGLE, J. J., and M. DELBRÜCK, 1951 Mutual exclusion between an infecting phage and a carried phage. J. Bacteriol. **62:** 301–318.

ZELLE, M. R., and J. LEDERBERG, 1951 Single-cell isolations of diploid heterozygous *Escherichia coli*. J. Bacteriol. **61:** 351–355.

ZINDER, N., and J. LEDERBERG, 1952 Genetic exchange in Salmonella. J. Bacteriol. **64:** 679–699